OPTIMIZATION OF BACTERIAL CELLULOSE PRODUCTION BY USING RESPONSE SURFACE METHODOLOGY (RSM): THE EFFECT OF PH, TEMPERATURE AND CONCENTRATION OF FERMENTATION MEDIUM.

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Abstract

Bacterial cellulose is a type of biopolymer that produced by *Acetobacter xylinum* in high purity, high water holding capacity, good mechanical strength, elasticity and high crystallinity. In this research, pineapple residue was used as the carbon sources to replace the pure carbon sources as the substrate for the synthesis of bacterial cellulose. The objective of this study was to investigate the effect of temperature, pH and concentration in the production of bacterial cellulose by *Acetobacter xylinum*. The important part in this research, including of preparation HS-Medium and agar plate as a medium for breeding the stock culture that was taken from Malaysia Agricultural Research and Development Institute (MARDI), Serdang, Selangor. Ideal condition in this research that investigated was varied from 40% to 100% for the concentration while the temperature was between 28°C to 32°C and pH were 4.5 to 8.5. Besides, this is study also aims to optimize the production of bacterial cellulose from pineapple residue using response surface methodology (RSM) based on the central composite design (CCD). Before RSM is used, the known value of the parameters was estimated based on one factor at that time (OFAT). The results obtained from the OFAT showed the optimum condition at pH 5.50, temperature 30°C and concentration of pineapple residue was 80 % where the amount of dry weight bacterial cellulose produced was 3.3948 g. According to the RSM result, the optimal set cultural conditions for bacterial cellulose were pH 5.15, temperature 30.51°C and concentration of pineapple residue was 83.32%. Bacterial cellulose production of 3.4368 g was achieved by using these optimal conditions. The existence of bacterial cellulose was proven by Fourier Transform Infrared (FT-IR) Spectroscopy analysis based on the appearance of absorbance peak for the C-C bonding, C-O bonding, C-OH bonding and C-O-C bonding. In addition, Scanning Electron Microscopy (SEM) was used to observe surface and cross section of the bacterial cellulose film. In short, the data presented in this paper showed that pineapple residue has a great potential as the carbon source in production of bacterial cellulose.

Abstrak

Selulosa bakteria ialah sejenis biopolimer yang dihasilkan oleh *Acetobacter xylinum* dengan ketulinan yang tinggi, keupayaan pegangan air, kekuatan mekanikal yang baik, keanjalan dan kristaliniti yang tinggi. Dalam kajian ini, sisa-sisa nanas digunakan sebagai sumber karbon untuk menggantikan sumber karbon tulen sebagai substrat untuk sintesis selulosa bakteria. Objektif kajian ini adalah untuk menyiasat kesan suhu, pH dan kepekatan dalam penghasilan selulosa bakteria oleh *Acetobacter xylinum*. Peranan penting dalam kajian ini termasuklah penyediaan medium HS dan plat agar sebagai medium untuk pembiakan stok kultur yang telah diambil daripada Institut Penyelidikan dan Kemajuan Pertanian Malaysia (MARDI), Serdang, Selangor. Keadaan ideal dalam penyelidikan ini yang disiasat telah di variasi dari 40% kepada 100% untuk kepekatan manakala suhu 28°C hingga 32°C dan pH 4.5-8.5. Selain itu, kajian ini juga bertujuan untuk mengoptimumkan penghasilan selulosa bakteria dari sisa-sisa nanas menggunakan kaedah respons permukaan (RSM) berdasarkan reka bentuk pusat komposit (CCD). Sebelum menggunakan RSM dalam penyelidikan ini, nilai yang diketahui daripada parameter-parameter telah dianggarkan berdasarkan *one factor at that time (OFAT)*. Keputusan yang diperolehi daripada OFAT menunjukkan keadaan optimum pada pH 5.50, suhu 30°C dan kepekatan sisa-sisa nanas adalah 80% di mana jumlah berat kering selulosa ialah 3.3948 g. Berdasarkan hasil RSM , syarat yang ditetapkan kultur optimum untuk selulosa bakteria pada pH 5.15, suhu 30.51°C dan kepekatan sisa-sisa nanas adalah 83.32%. Penghasilan selulosa bakteria dengan 3.4368 g telah dicapai dengan menggunakan syarat-syarat yang optimum ini. Kewujudan selulosa bakteria telah dibuktikan oleh analisis *Fourier Transform Infrared (FT-IR) Spectroscopy* berdasarkan kemunculan puncak absorbansi bagi ikatan C-C, ikatan C-O, ikatan C-OH dan ikatan C-O-C. Tambahan pula, *Scanning Electron Microscopy (SEM)* telah digunakan untuk memerhati permukaan and keratan rentas daripada kepingan selulosa bakteria. Kesimpulannya data yang diperolehi ini menunjukkan bahawa sisa-sisa nanas mempunyai potensi yang besar sebagai sumber karbon dalam penghasilan selulosa bakteria.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

BC	Bacterial Cellulose
°C	Degree C
C	Carbon
C_3	Carbon 3
C_6	Carbon 6
cm	Centimetre
g	Gram
ε	Error
IR	Infrared
ml	Millilitre
Y	Dry weight of Bacterial Cellulose
%	Percentage

LIST OF ABBREVIATIONS

Adj R^2	*Adjusted R^2*
ANOVA	Analysis of variance
CCD	Central composite design
DOE	Design of experiment
Et al	An others
FTIR	Fourier Transform Infrared
MARDI	Malaysia Agricultural Research and Development Institute
$MgSO_4.7H2O$	Magnesium Sulphate Heptahydrate
NaOH	Sodium hydroxide
Na_2HPO_4	Disodium hydrogen phosphate
OFAT	One factor at a time
pH	Potentiometric hydrogen ion concentration
RSM	Response surface methodology
SEM	Scanning electron microscopy

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

Cellulose is the most abundance polymer that is present as the major component of plant biomass and a representative of microbial extracellular polymers. *Acetobacter xylinum* is the bacteria that able to grow on the waste material to produce cellulose. The several genera that has shown the ability to synthesize cellulose include *Sarcina, Agrobacterium, Rhizobium and Acetobacter* (Barbara *et al*., 2008). However, species that able to produces cellulose in high quantity is *Acetobacter xylinum* and over a century ago this organism and its produce were first identified based on the research (Iguchi and Yamanaka, 1997).

Stationary culture, agitated culture, cultivation in the horizontal fermenters or cultivation in the internal-loop airlift reactors are the few techniques that have been reported for economic and commercial bacterial cellulose production (Prashant *et al*., 2009). The monosaccharide or simple sugar such as glucose, xylose and glucose that act as a substrate (Ishihara *et al*., 2002) or other carbon sources such as ethanol and glycerol are the sources in a medium for bacterial cellulose production (Sherif and Kazuhiko, 2005). *Acetobacter xynlinum* synthesizes cellulose by fully utilizing the monosaccharide of carbohydrate or simple sugar such as glucose, fructose, sucrose or lactose.

Besides that bacterial cellulose has application in paper, textile and food industries and also as a biomaterial in cosmetic and medicine (Ring *et al*., 1986). Synthesis of bacterial cellulose is one of the methods in producing cellulose biopolymers. Another popular method is by extracting and isolating the cellulose from

plant but synthesis of bacterial cellulose is more popular because time consumption to produce is shorter than plant synthesis (Klemn *et al*., 2001) thus it also has the chemical purity as one of the most important features of bacterial cellulose that differentiate it from the plant cellulose (Surma *et al*.,2000).

The aim of this study is to optimize the bacterial cellulose production by using pineapple residue as a substrate that consists of pineapple core, the peeling skin and the pineapple crown. In this experiment, three parameters are used, which are pH, temperature and concentration of pineapple residue are evaluated with intention to find the optimum condition to generate the maximum mass of bacterial cellulose.

1.2 PROBLEM STATEMENT

Annually, there are approximately 15000 tonnes of pineapple residues produced from the pineapple cannery industries in Malaysia. The pineapple residue left over from the production processes are abundant and still contain the large amount of sugar content (Akhiro *et al*., 2008). Usage of pineapple residue as raw material in bacterial cellulose production not only reduces waste material created from the pineapple industry, but also lowers the cost of bacterial cellulose production. In addition, the used of pineapple residue as raw material in bacterial cellulose production also can prevent from the environmental issues. Besides that, most cellulose is obtained from the plant cell wall is not pure, and it is difficult to purify the cellulose from lignin and hemicelluloses (Klemn *et al*., 2001). Bacterial cellulose is used as an alternative instead of plant cellulose in order to produce high purity cellulose and to reduce the forest depletion (Barbara *et al*., 2008). Pineapple residue is used in this research as a raw material of carbon source in order to reduce the peel, crown and core from pineapple that discharged from food and beverage industries.

The carbon sources in pineapple residue consist of sucrose, glucose, fructose and other nutrients (Sasaki *et al*., 1991; Krueger *et al*., 1992). Therefore, it can be consumed to produce the value added product such as bacterial cellulose (Abdullah and Hanafi, 2008).

1.3 OBJECTIVES OF RESEARCH

i) To investigate the effect of temperature, pH and concentration of pineapple residue in the production of bacterial cellulose by *Acetobacter xylinum*.
ii) To optimize the production of bacterial cellulose from pineapple residue by using response surface methodology (RSM)

1.4 SCOPES OF RESEARCH

In order to achieve the objective, scope of study was divided into three as the following:

i) To produce bacterial cellulose from pineapple waste residue as a fermentation medium with different temperature, pH and concentration.
ii) To optimize the parameter by using response surface methodology (RSM)
iii) To analyse the bacterial cellulose produced by using FTIR.
iv) To characterize the morphology of the bacterial cellulose by using Scanning Electron microscope (SEM).

1.5 RATIONAL AND SIGNIFICANT OF STUDY

i) Reuse pineapple residue that produced from food and beverage industries to produce bacterial cellulose.
ii) Low cost bacterial cellulose production.
iii) High productivity of bacterial cellulose production

CHAPTER 2

LITERATURE REVIEW

2.1 BACTERIAL CELLULOSE

Bacterial cellulose is a polymer that produced by *Acetobacter xylinum* in presence of glucose. Bacterial cellulose has high purity cellulose where it was free from lignin and hemicelluloses not like plant cellulose that has low purity cellulose and containing lignin and hemicelluloses (Klemn *et al*., 2001). There are several aspects that differentiate bacterial cellulose with plant cellulose, which are bacterial cellulose has unique characteristic, including good mechanical strength, high water absorption capacity, high crystalline, ultra-fine and highly pure fibre network structure that caused bacterial cellulose more preferred than plant cellulose (Keshk and Sameshima, 2006). There are four different pathways in forming the cellulose biopolymer (Klemn *et al*., 2001). The first pathway is by the isolation of cellulose from plants. This pathway involved another separation process step to remove lignin and hemicelluloses. The second pathway is the synthesis of cellulose by *Acetobacter xylinum*. In the synthesis process of cellulose, bacteria that can produce the highest cellulose amount than other bacteria is *Acetobacter xylinum. Acetobacter xylinum* produced cellulose in the form of the extracellular pellicle composed of ribbons while *Achromobacter, Aerobacter, Alcaligenes* produce cellulose in fibrils form, *Agrobacterium* and *Rhizobium* produces cellulose in the form of short fibril, *Pseudomonas* produce bacterial cellulose with no distinct fibril, *Sarcina* produce an amorphous cellulose and *Zoogloea* produce cellulose in not a well defined form (Barbara *et al*., 2008). The third and fourth methods are by the first enzymatic in-vitro synthesis starting from cellobiosyl fluoride and the first chemosynthesis from glucose by ring opening polymerization of benzylated and pivaloylated derivatives (Klemn *et al*., 2001).

Cellulose is the main part in the cell wall plant and act as protective and coating, whereas plant cellulose (PC) plays a structural role in plant (Bielecki *et al.*, 2000). However cellulose is obtained in the plant is not pure caused it have lignin and hemicellulose, so the separation process to remove lignin and hemicellulose is the most popular and industrial important isolation of cellulose from plants (Klemn *et al*., 2001), but it is difficult to purify the cellulose from lignin and hemicelluloses through the separation process. Nowadays, bacterial cellulose used as an alternative instead of plant cellulose in order to produce high purity cellulose and in the same time to reduce the forest depletion (Sherif, 2008). Most of the paper production used cellulose pulp from plant and thus gives a problem on forest depletion and now many researches has been conducted on producing paper from bacterial cellulose and as a result, there is an improvement of the paper's strength properties and protect the surface of paper (Barbara *et al*., 2008). The form of its size, crystallinity and purity had differentiated between bacterial cellulose (BC) produced by bacteria that has unique physical and chemical properties with cellulose that produced from plant (Prashant *et al*., 2008). Bacterial cellulose also has unique characteristic, including good mechanical strength, high water absorption capacity, high crystallinity, ultra-fine and highly pure fibre network structure that caused it has been preferred than plant cellulose (Andelib and Nuran, 2009).

Bacterial cellulose also has disadvantages that need to encounter although bacterial cellulose has a unique characteristic than the plant cellulose, the problem is the price for the sugar as a substrate is very expensive but low in the quantity production of the process. Using the pineapple residue and fruit waste such as fruit peel is one of the alternatives that can overcome this problem. The mango peel, pineapple core, watermelon peel and other fruit wastes are the example of fruit waste that can be utilized as substrates to produce cellulose (Akihiro *et al*., 2008). Besides that lactose which has a lower price also potential to be used as a substrate for cellulose production using static culture.

2.2 WASTE AS A SUBSTRATE

One of the advantages bacterial cellulose is it can produce from various carbon and nitrogen sources. Various carbon sources including D-glucose, sucrose, fructose, D-galactose, lactose, mannitol and ethanol while for various nitrogen sources are ammonium sulphate, ammonium nitrate, Riboflavin, Glycine, Peptone, sodium nitrate and Methionine (Panesar *et al*., 2009). Carbon and nitrogen sources played important role for cell growth and bacterial cellulose production, and in the same time cost for bacterial cellulose production must be considered as a main objective. Traditionally, bacterial cellulose production that using pure glucose as a carbon source and other nutrient sources was resulting in very high production cost. One of the solutions to overcome this problem by using waste as a substrate to replace pure glucose and that was proven by previous research about waste effective in bacterial cellulose production. Using cheap carbon and other nutrient sources such as from agro-forestry industrial residues is an interesting strategy to overcome the high production cost. Many of researchers were applied various wastes in bacterial cellulose production by using beet molasses (Kesk *et al*., 2006), sugar cane molasses and corn steep liquor (El-saied *et al*., 2008), several fruit juices, including orange, pineapple, apple, Japanese pear and grape (Kurosumi *et al*., 2009), pineapple waste (Ch'ng and Muhamad, 2000) and coconut water (Kongruang, 2008) was already successfully used as carbon sources for the bacterial cellulose production. An addition the value of industrial waste will have added value and give positive impact by reducing waste material in the environment for bacterial cellulose production (Pedro *et al*., 2011).

2.3 MEDIUM CONDITION

Medium condition was played as a main role for ensure bacterial cellulose production successfully. In production of bacterial cellulose using *Acetobacter xylinum*, temperature needs to be maintained at 30^{o}C and pH will be measured at 5.5 by pH meter to ensure optimized growth of this microbe, that gives the highest dry weight of bacterial cellulose (Pourramezan *et al*., 2009). Normally, the previous research, most of the researchers study the effect concentration of glucose into the fermentation medium, but in this research study the effect concentration pineapple residue to replace pure

glucose as a parameter into the medium. Based on reported Son *et al*., 2003, was mentioned that increasing amount of glucose into the medium will enhance bacterial cellulose production but the yield will decrease when medium containing more glucose excess. In this research, concentration was varied with 40%, 50%, 60%, 70%, 80%, 90% and 100% as a percentage for pineapple waste concentration and supported by the other nutrients for *Acetobacter xylinum* growth. Based on the previous research during the fermentation process, the pH of the medium change throughout the process. This caused by side product was produced during conversion of glucose to cellulose by synthesis of *Acetobacter xylinum*. This microbe also converted glucose in pineapple waste to gluconic, lactic and acetic acid as a side product. These side products will be accumulated and affecting the condition of the culture medium thus decrease the bacterial cellulose production (Chawla *et al*., 2008).

2.4 BACTERIAL CELLULOSE SYNTHESIZE

Cellulose is the most abundant earth biopolymer and also known as the major component of plant biomass and a representative of bacterial polymers in extracellular condition. An efficient producer of cellulose are acetic acid bacteria *Acetobacter xynilum*. Bacterial cellulose free of lignin and hemicelluloses (Barbara *et al*., 2008). In addition, with extracellular synthesized bacterial cellulose will be different with plant cellulose with respect to its high crystallinity, high water absorption capacity, and mechanical strength in the wet state and ultra fine network structure (Budhiono *et al*., 1999). The ability to produce high levels of polymer in a large range of carbon and nitrogen sources that caused *Acetobacter xylinum* has applied as a model for the basic and was applied studies on cellulose. The precisely and specifically synthesis of bacterial cellulose was regulated multi step process, that mean involving a large number of both individual enzymes and complexes of catalytic and regulatory proteins, whose supramolecular structure has not yet been defined (Klemn *et al*., 2001). The cellulose formation includes five fundamental enzymes mediated steps: the transformation of glucose to UDP-glucose via glucose-6-phosphate and glucose-1-phosphate and finally, the addition of UDP-glucose to the end of a growing polymer chain by the cellulose synthase (Prashant *et al*., 2008). The overall mechanism for cellulose biosynthetic pathway is illustrated in Figure 2.1.

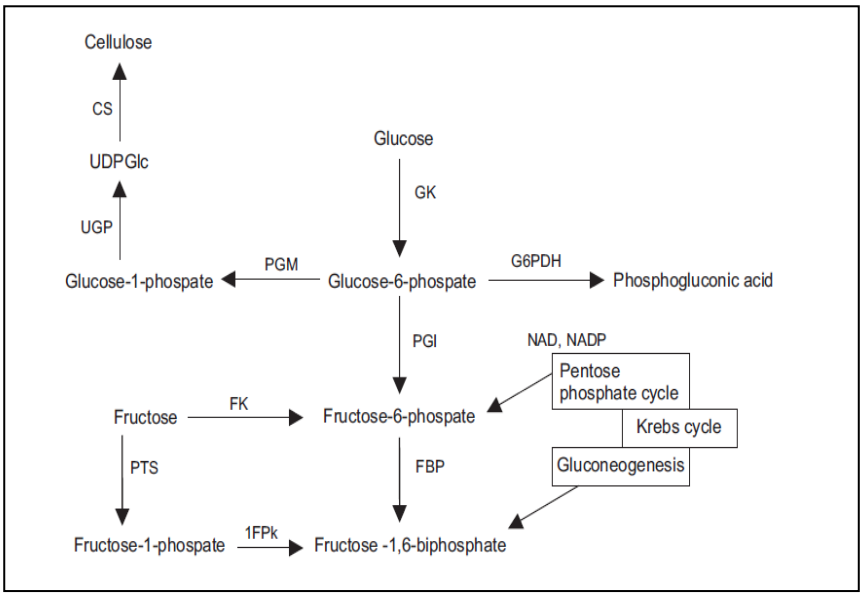

Figure 2.1: Simplified model for the biosynthetic pathway of Bacterial Cellulose.

Sources: Prashant *et al*., 2008

2.5 *ACETOBACTER XYLINUM*

Acetobacter xylinum is a gram negative microbe that produced bacterial cellulose in aerobic condition. *Acetobcater xylinum* also acetic microbe that growth very well in acid condition from broth culture and involve in fermentation process to convert glucose to cellulose. Gluconic, acetic or lactic acid is produced by *Acetobacter xylinum* in fermentation process caused the pH decrease from pH 6 to pH 4 in culture medium and at the same time the yield of cellulose decrease in fermentation (Chawla *et al*., 2008) but *Acetobacter xylinum* still growth because it is a type of acetic microbe. In alkaline condition, *Acetobacter xylinum* will grow slowly and bacterial cellulose yield will decreasing (Pourramezan *et al*., 2009). *Acetobacter xylinum* can produces cellulose from a variety of carbon sources including glucose, ethanol, sucrose, and glycerol and it produced cellulose in the form of extracellular pellicle composed of ribbons. This microbe can be obtained from the nectar of flowers, decaying fruit, fresh apple cider and unpasteurized beer which have not been filtering sterilized (Barbara *et al*., 2008).

2.6 PINEAPPLE RESIDUE

2.6.1 Pineapple Canning Industry

Malaysian Cannery of Malaysia Sdn. Bhd is a location to obtain the pineapple residue. The canning factory is the first place for the fresh pineapple fruits to be submitted. After that the fruits will be graded into several sizes according to the fruit diameter. Then they will be peeled, core removed, sliced, sorted and canned. All the peeled skin, unwanted fruits and the core will be sent to the crush machine for crushing. After crushing, the solid waste will be sent to cattle feeding and in the same time the liquid waste is send to storage for fermentation process (Abdullah and Hanafi, 2008). Figure 2.2 shows the pineapple canning process.

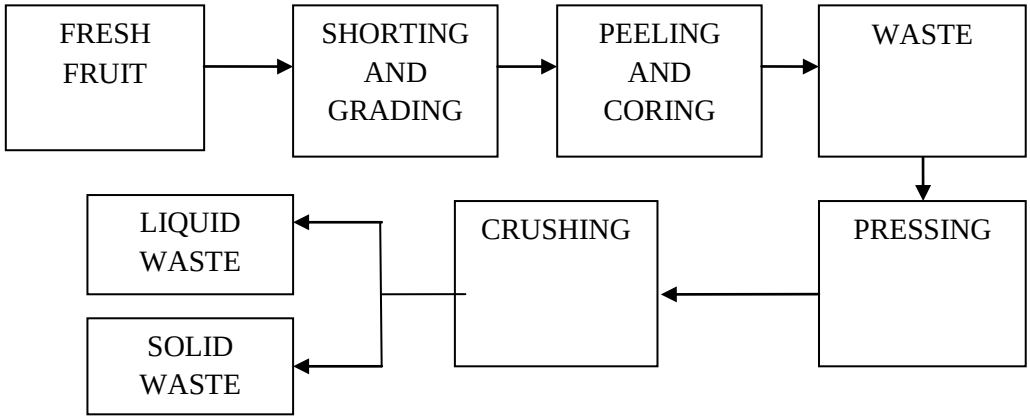

Figure 2.2: The pineapple canning process

Sources: Abdullah and Hanafi Mat 2000

2.6.2 Pineapple Residue Characteristics

Primarily solids in the form of peels, stem, pits, culls and organic matter suspension are the wastes that generated by fruit processing. Identify and characterise the wastes either solid or liquid that were produced is the first stage in the optimization of waste reduction. Each particular food industry generates specific type and amount of waste. For example, more solid waste were generated by the fruit and vegetables industry than the dairy industry. The problem of suspended organic matter in the waste

water is the characteristics of the waste load of various fruit processing industries (Moon and Woodroof, 1986). The comparison between pineapple waste to other fruit processing wastes is given in table 2.1. The solid waste from pineapple processing was about 45% from fresh fruit, followed by citrus, apple, pear, peach and cherry were 43,32, 30, 24, 17, and 14% respectively. The suspended and organic matter in the waste water is higher than other fruits processing for pineapple processing. It can be indicated by the BOD and suspended solid contained in the rinse water which are 4.8 kg/m^3 and 2.4kg/m^3 respectively.

Table 2.1 : The comparison between pineapple waste to other fruit processing waste.

Fruit	Raw (tones)	Waste water (m^3)	BOD (kg/m^3)	Suspended solid (kg/m3)	Solid residual (tonnes)
Apple	1,000,000	18,920,000	0.95	0.11	320,000
Apricot	120,000	2,270,000	1.39	0.20	21,000
Cherry	190,000	1,130,000	1.60	0.40	27,000
Citrus	7,800,000	87,050,000	0.16	0.28	3,390,000
Peach	1,100,000	16,650,000	1.79	0.29	270,000
Pear	400,000	6,050,000	2.09	0.74	120,000
Pineapple	1,000,000	1,890,000	4.80	2.40	450,000

Sources: Moon and Woodroof (1986)

The characteristics of solid waste from pineapple processing are shown in table 2.2 reported by different authors. The moisture content of solid waste was found to be at the range of 87.50-92.80%. The difference of moisture content might be due to the sample obtained from various geographical origins and of varying degree of ripeness. The total nitrogen and ash content in the wastes were between 0.90-0.95% and 3.9

10.6% respectively. Pineapple that consist of rind, crown and core that prepared in juice form is one of the unconventional media indentified that can promote a low cost substrate for the production of bacterial cellulose. Although the amount of sugars in the pineapple rind, crown and core is much lower than the total sugar in the pineapple flesh, it still can act as a carbon source for *Acetobacter xylinum* to produce bacterial cellulose. The cost of collecting the pineapple rind waste is much lower than buying the pure glucose medium for the cellulose production and these wastes can caused environmental pollution problems if it not is utilized.

Table 2.2: The characteristics of solid pineapple waste reported by different authors.

Composition (%)	Bardiya et al. (1996)	Viswanath (1992)	Chandapillai and Selvarajah (1978)
Moisture	92.80	87.69	89.70
Total solid	7.80	12.31	10.30
Ash	10.60	6.20	3.90
Organic carbon	51.85	38.9	-
Nitrogen free extract	-	-	75.10
Total carbohydrates	35.00	-	-
Ether extract	-	-	0.20
Cellulose	19.80	-	-
Crude fibre	-	-	14.70
Hemicelluloses	11.70	-	-
Phosphorus		0.08	0.10
Total soluble	30.00	-	-
Total nitrogen	0.95	0.90	-

Sources: Abdullah and Hanafi Mat (2000)

2.7 PURIFICATION OF BACTERIAL CELLULOSE

The purification is the important step in the production of any cellulose product, which is traditionally in the paper making industry known as chemical pulping process. This process is aimed to remove essentially all of the undesired residual insoluble lignin and other chemical bound to cellulose fibre as well as impurities occurring during processing and converting them into compounds which are soluble in alkaline water. The photo-oxidation that caused discolouration probably will occur if lignin is not removed and the final paper product is fragile (Saharman *et al*., 2011). Therefore various method have been developed and different chemical have been used to produce a good quality paper as well as to overcome low pulp yields (Bajpai, 2005). Plant cellulose as a naturally is bound with lignin and hemicelluloses and also other chemicals but bacterial cellulose is not bound to other chemicals and it has high purity (Klemn *et al*., 2006). Therefore the purification process that was applied on the bacterial cellulose differs from plant cellulose purification.

There are two stages in the purification process, firstly bacterial cellulose was washed in 2.5wt% NaOH solution for one day and then washed with 2.5wt% NaOCI also for 1 day (Saharman *et al*., 2011) and it had known as the two step purification process. An addition using 2.5wt% NaOH solution will prevent lower mechanical properties in bacterial cellulose but using 6% NaOH can potentially change the crystal structure of bacterial cellulose (Gomes *et al*., 2007) by breaking of many inter and intra molecular hydrogen bonds, which are naturally present in cellulose. Protein and nucleic acid that were derived from bacterial cellulose, and the culture broth were known as the non cellulose material will be removed from the pellicle by using two step purification process and in the same time the pellicle will form inter and intra fibrillar hydrogen bonds that strong. Normally, within a few weeks for untreated bacterial cellulose sheet can present mould on the surface and the result it changes the colour and becoming darker but bacterial cellulose that was experience two step purification it can stored for a long time and without change in colour and quality. The importance for two step purification process is to allow observation easily the internal structure bacterial cellulose using FT-IR and it show that NaOCI as a bleaching agent is effectiveness in removing impurities, which are not removed with NaOH alone (Saharman *et al*., 2011).

2.8 APPLICATION OF BACTERIAL CELLULOSE

Nowadays, in new commercial application of secondary and third degree burn, bacterial cellulose will be shown to be very successful in the medical treatment (Prashant *et al*., 2008). To prove this triumph a clinical study has been performed on 34 patients and in this analysis the bacterial cellulose wound dressing materials were directly applied on the fresh burn covering up to 9-18% of the body surface. Then macroscopic observation of the wound and wound extract, epidermis growth, microbiological test, and histopathological are diagnoses that were considered in this clinical study. The result is bacteria cellulose is to be one of the best material to promote wound healing from burns caused a moist environment for tissue regeneration, specific cellulose nano-morphology which promotes cell interaction and tissue re-growth and significant reduction of scar tissue formation (Prashant *et al*., 2008). Bacterial cellulose also is used in wound care such as surgical wounds, ulcers, tissue and organ engineering and it can be used in various areas including pharmaceutical and waste treatment (Prashant *et al*., 2008).

The chemically pure cellulose in bacterial cellulose is also applied in food application to food process as thickening and stabilizing agent (Prashant *et al*., 2008). In 1992, bacterial cellulose is used into diet drinks in Japan and the first use of bacterial cellulose in the food industries was in nata de coco in the Philippines (Budhiono et *al*., 1999).

The application of papermaking using bacterial cellulose is also popular in the paper industry (Surma *et al*., 2008). Cellulose pulp from plant were used in most of paper production and thus gives a problem on forest depletion. As a result to improve of the paper's strength properties and protect the surface of paper (Surma *et al*., 2008) and in the same time to reduce forest depletion, many researches has applied bacterial cellulose in producing paper.